Thermal inactivation of bacterial spores using a precision temperature control system

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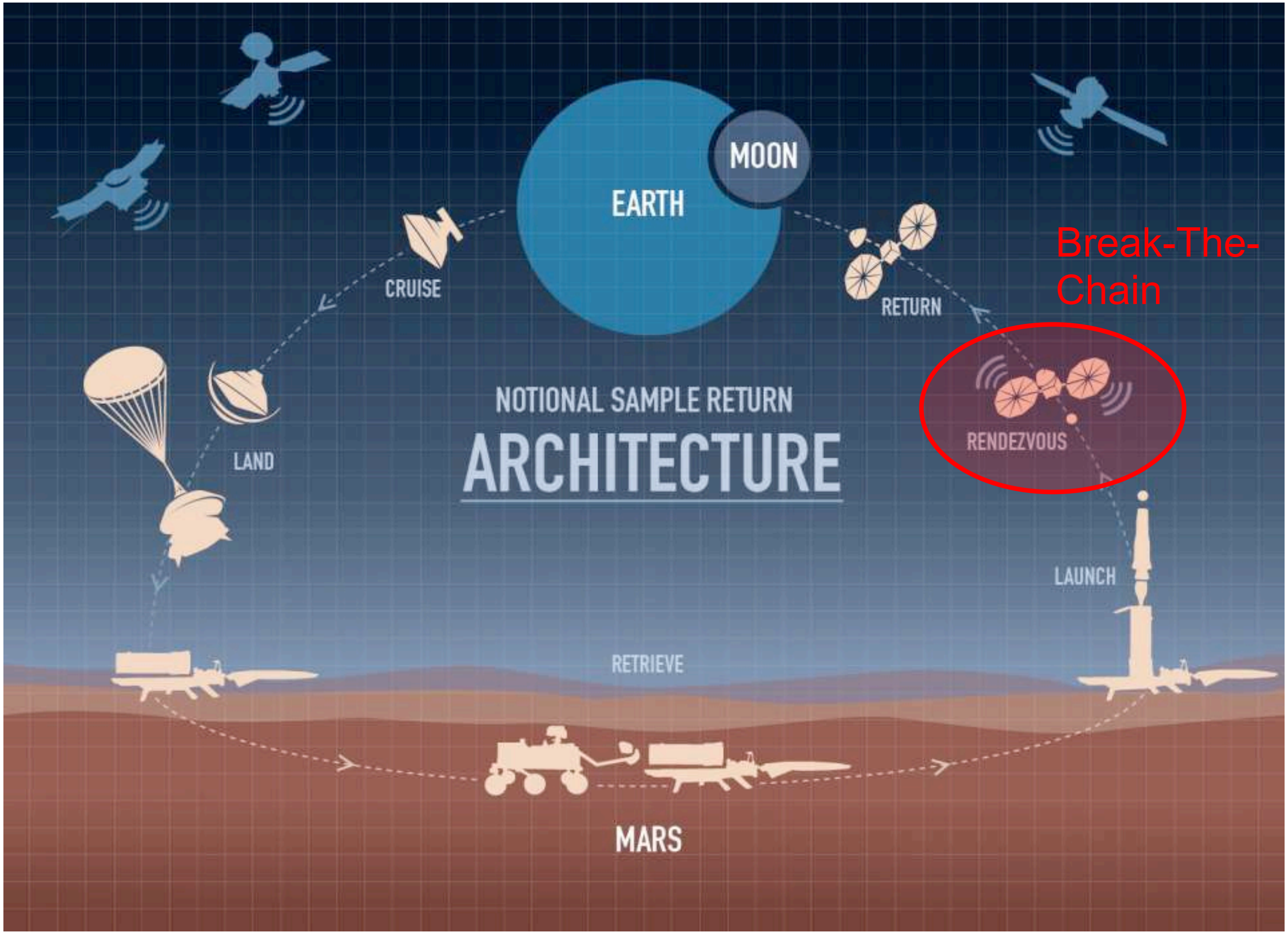
Concept for Sample Return



Pre-decisional: for information and discussion only.

NASA HQ, Notional MSR Slide, Zurbuchen

Evaluation for a specific step in breaking-the-chain for returning Mars Samples

- The current notional Mars sample return architecture calls for means to keep surfaces exposed to Mars conditions and Mars dust separated from the hardware that would return to Earth.
- A double-walled sample containment system will provide a physical barrier to most of the sample return container, except a small zone where the container is to be sealed.
- The current baseline plan is to seal the container by a brazing process.
- Brazing has a wide range of possible temperatures (300 – 700°C).
- This work was undertaken to understand if the lower temperatures are good options for a brazing process with respect to possible contamination by viable microbes.

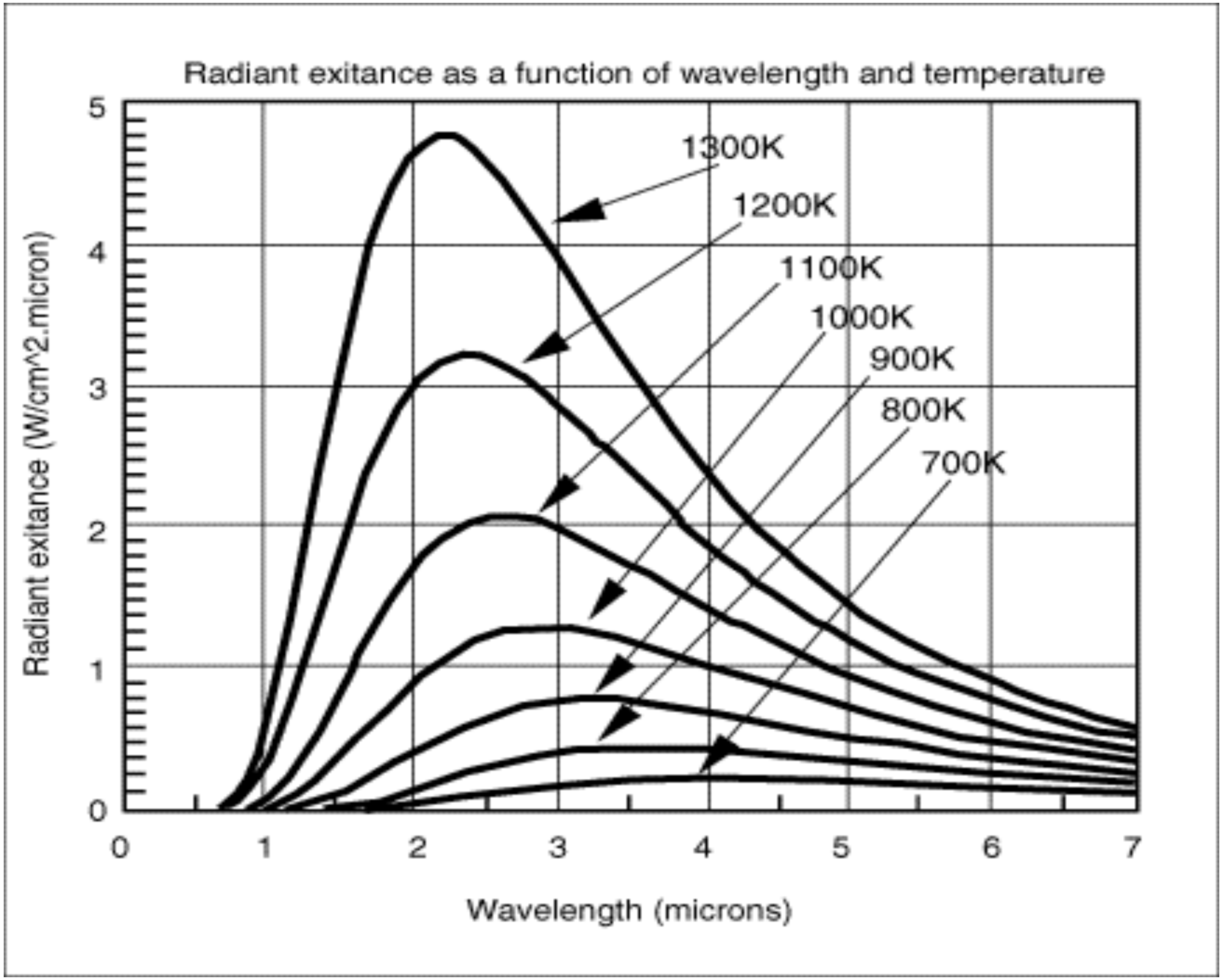
Approach for a single step in breaking-the-chain

- Our approach was to expose biological indicators to similar heating profiles anticipated from brazing processes.
- Sealing a Mars Sample Container is expected to be a rapid application of heat to Mars exposed surfaces.
- An apparatus previously designed for rapid heating was refurbished for this work.

Precision Heating Apparatus Design Considerations

- Provide a method to control the heat exposure and temperature profile to model anticipated rapid heating scenarios
- Control complex-shaped heating profiles for temperatures from ambient to 500°C
- Heating by tungsten-halogen lamps
- Temperature of the substrate monitored in each run
- Capability to use vacuum or special atmospheric gas mixes

Planck's curve describing the density of electromagnetic radiation emitted by a blackbody



Merchant, John. "Infrared Temperature Measurement Theory and Application."
OMEGA Engineering

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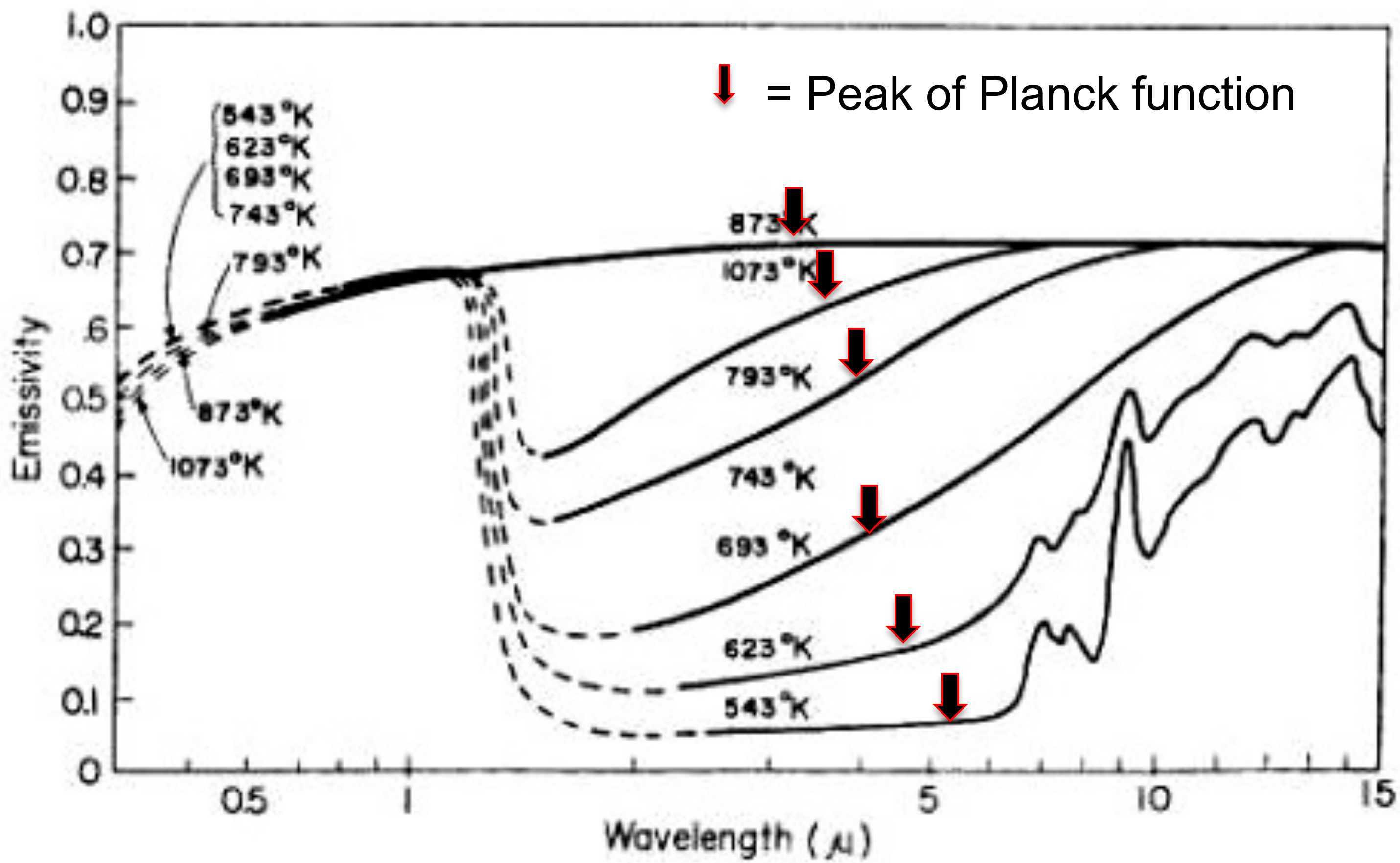
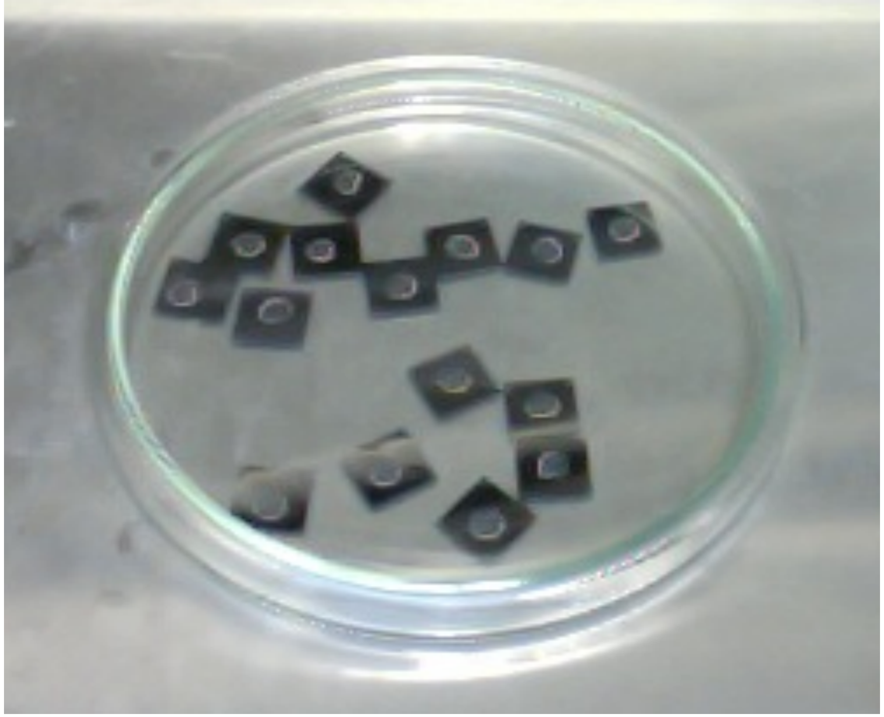
Emissivity of Silicon



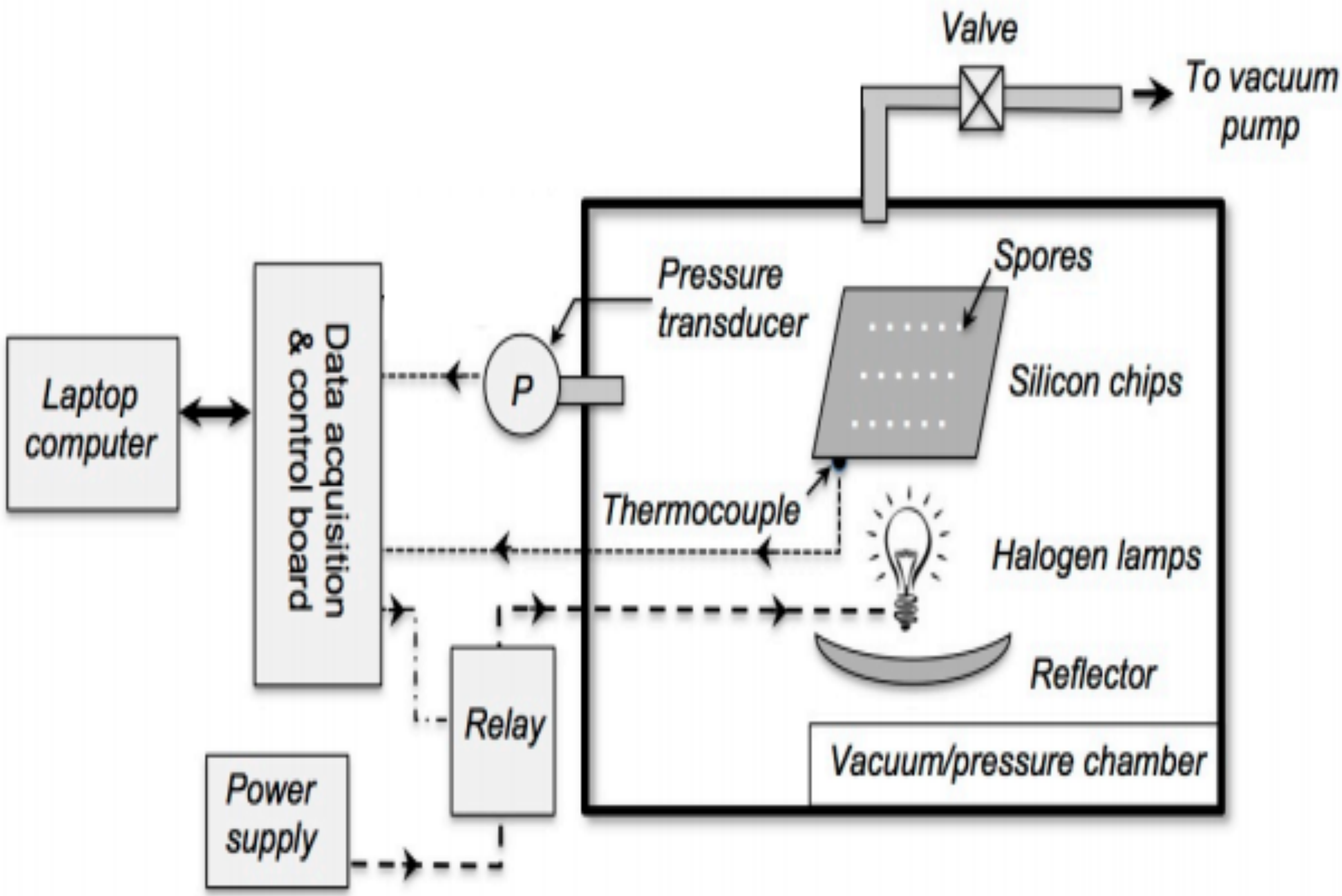
Emissivity of silicon as a function of temperature and wavelength (T. Sato, *Jap. J. Appl. Phys*. 6, 339-347 (1967)).

Silicon wafer substrate

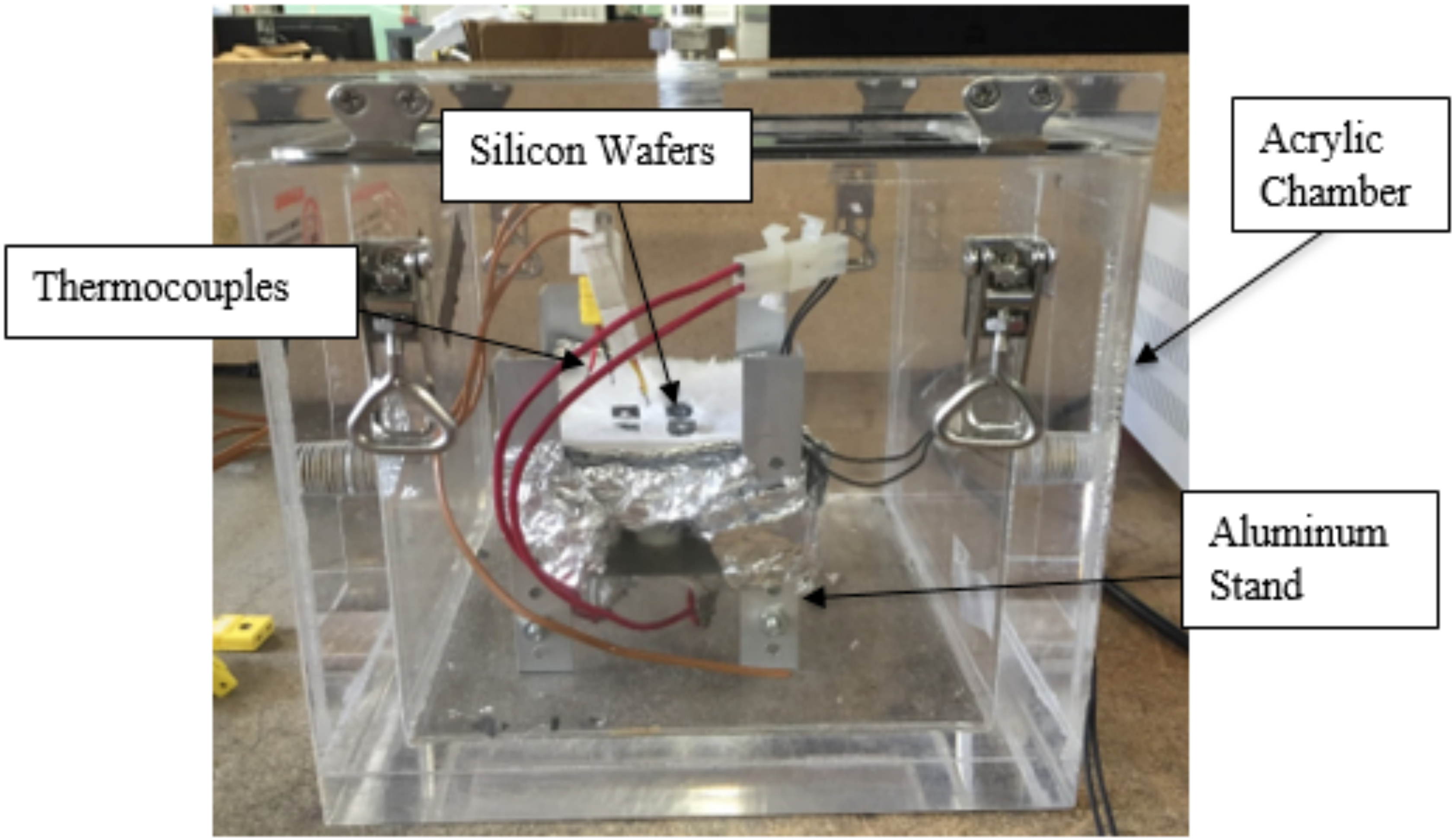
- Features:
  - 100 uM (very thin) so permits rapid heating
  - Thermal conductivity is 10x that of stainless steel so permits even heating
  - Emissivity Profile minimizes heat loss
  - Biologically inert
  - Smooth flat surface



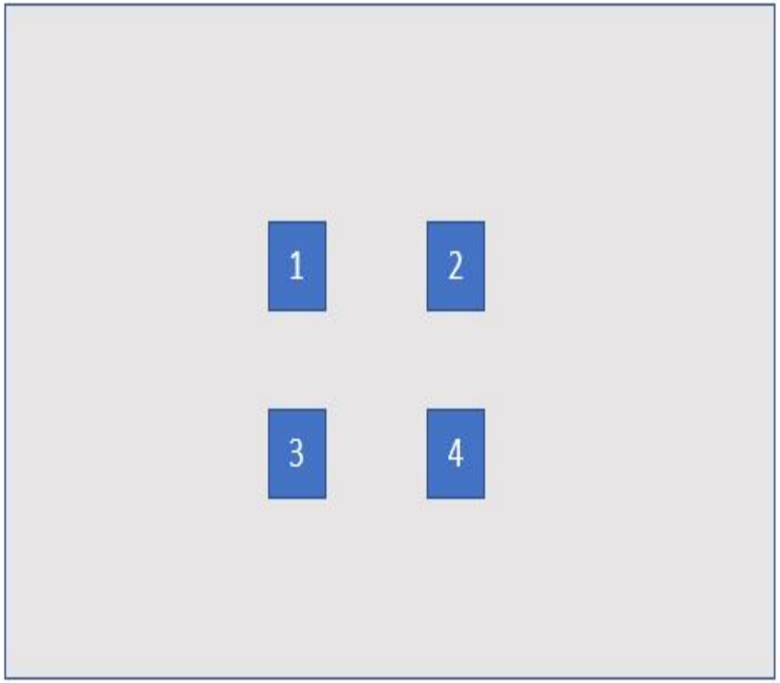
System Diagram



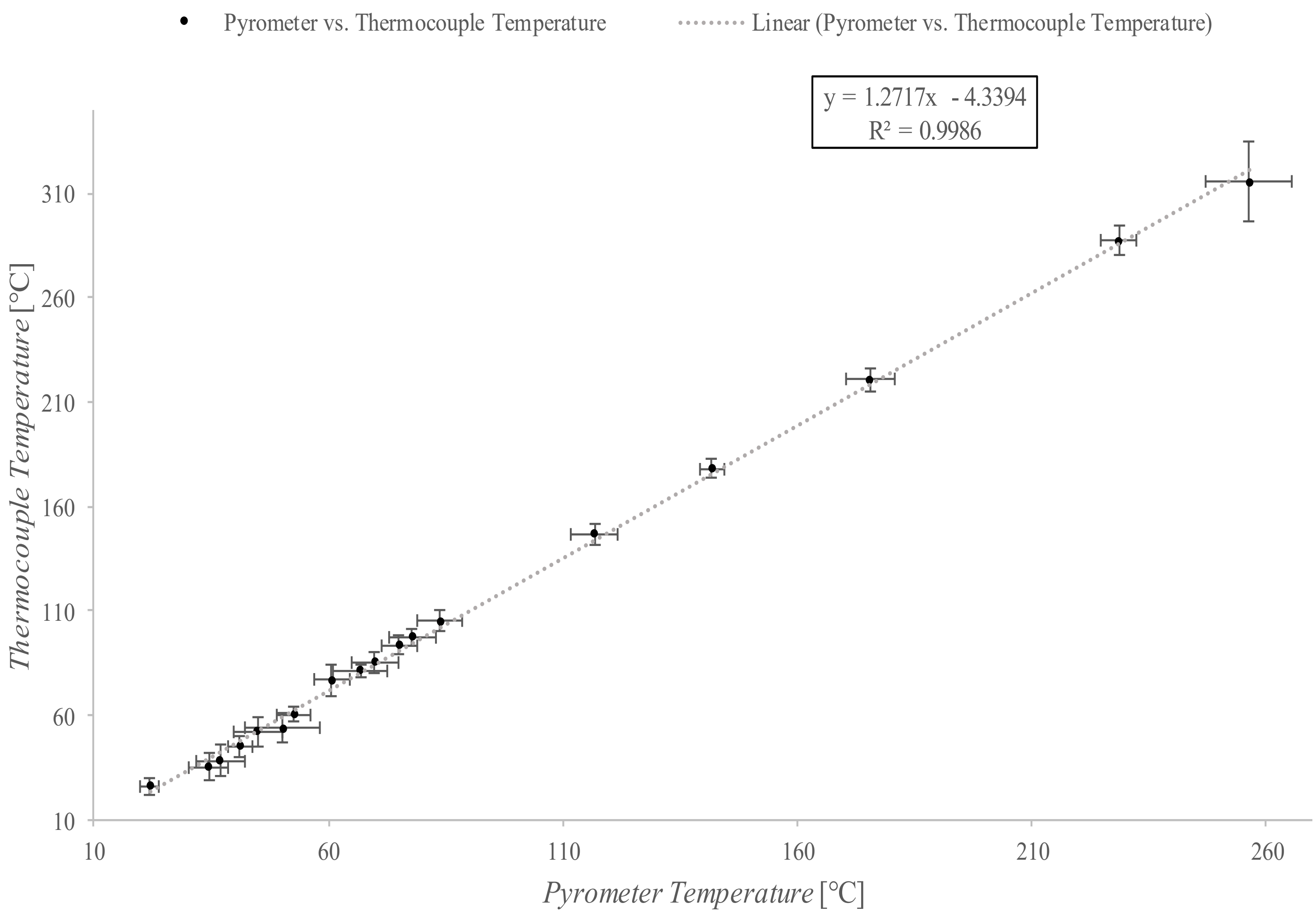
Precision Heating Apparatus



Sample exposure platform



Pyrometer & Thermocouple Calibration



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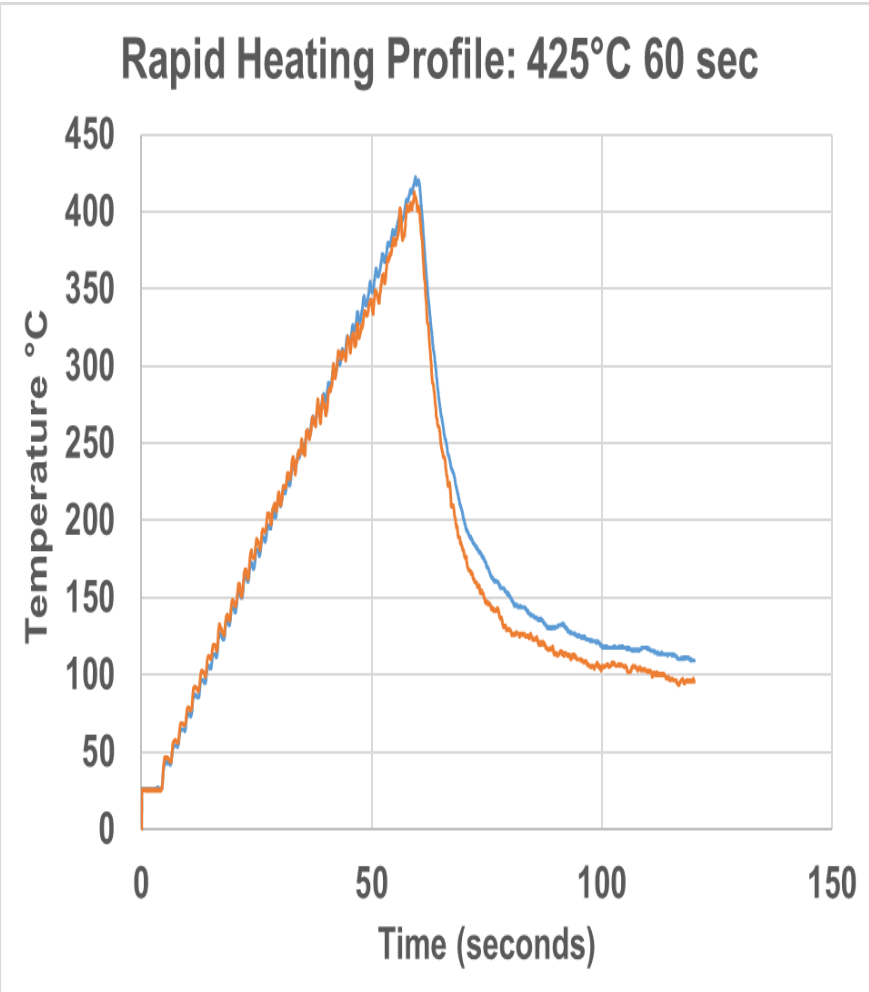
Selection of Test Organisms

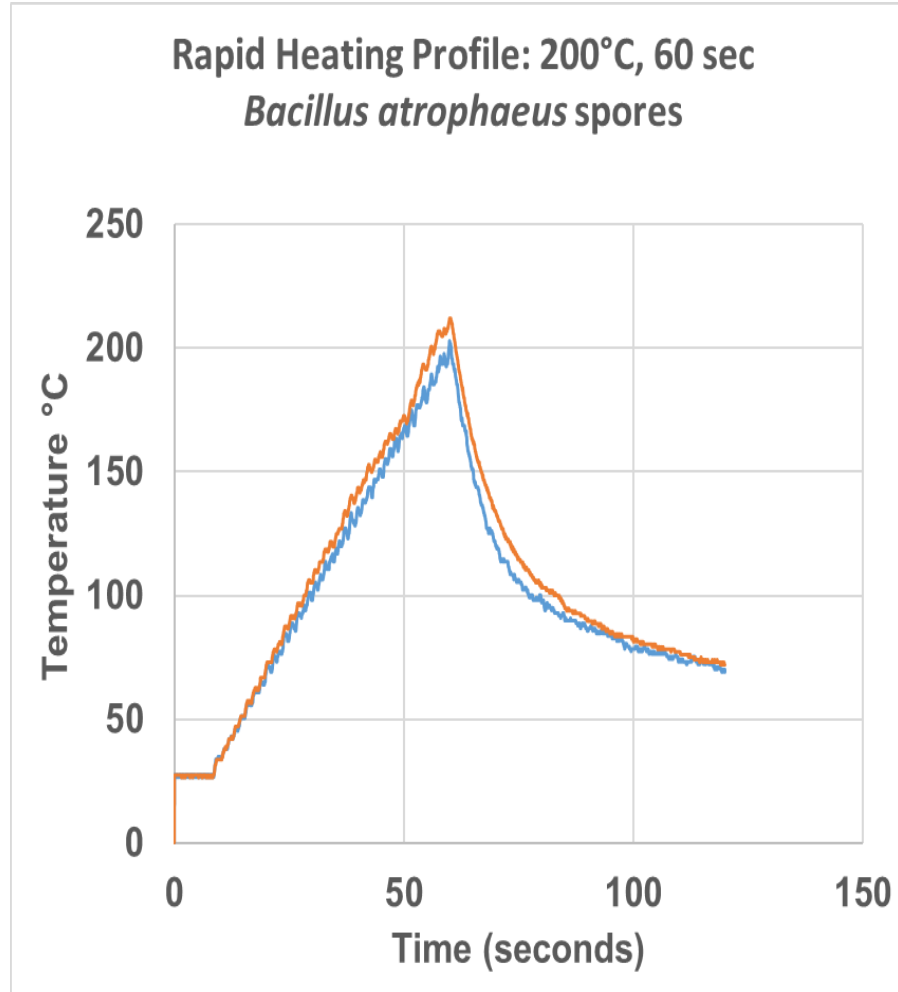
- Bacterial endospores are very heat resistant and serve as a surrogate to demonstrate the effectiveness of the heating process.
- Spores from strains selected for this study:
  - *Bacillus atrophaeus* ATCC 9372
    - Standard biological indicator strain for heat microbial reduction
  - *Bacillus* sp. ATCC 29669
    - Most Heat resistant spore for testing in our collection
    - Viking-era isolation from KSC
    - Considerable experience with this spore

Design of Heating Profiles

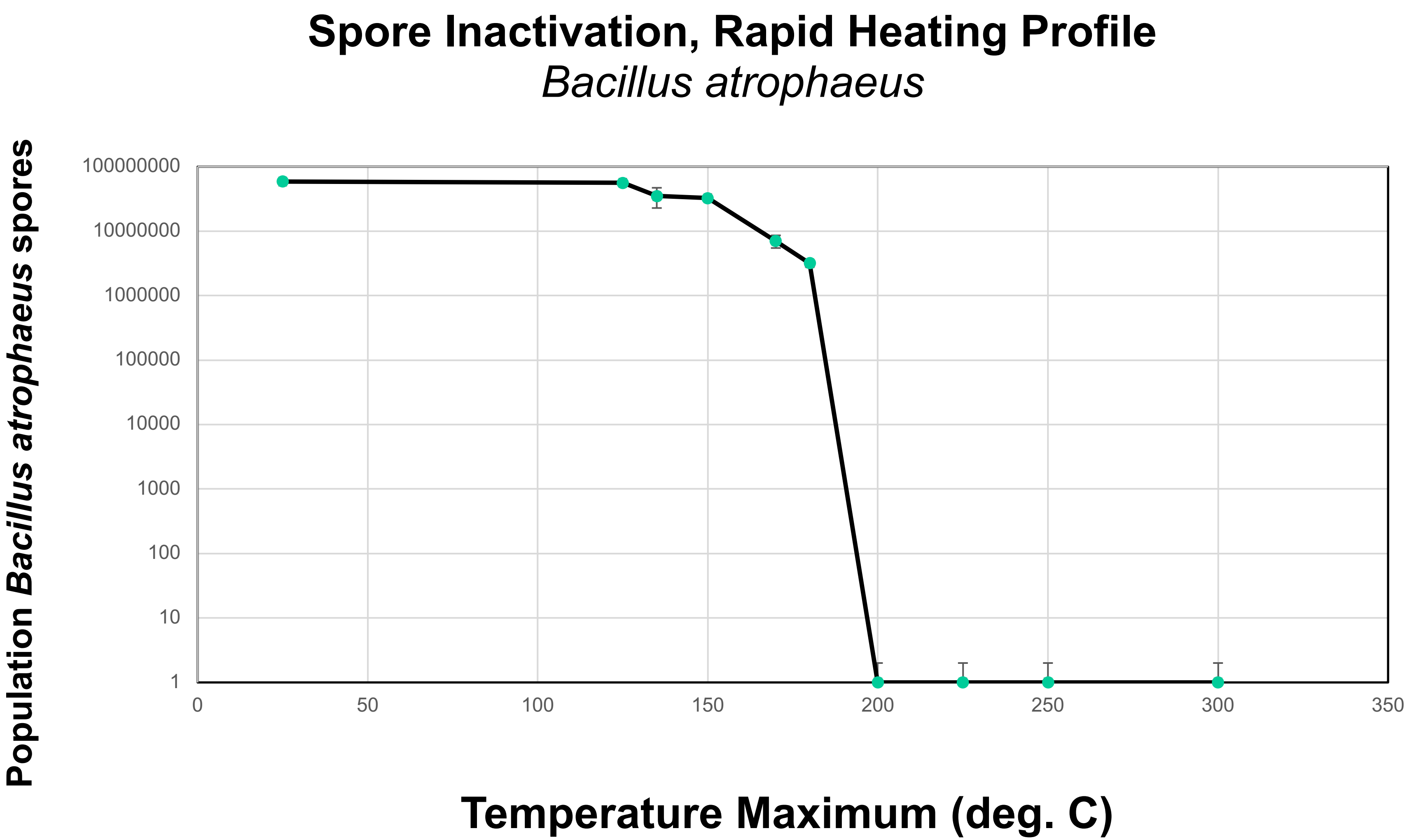
- The sealing techniques likely to be employed will probably be very quick, perhaps less than a minute heat up time.
- An apparatus originally designed for testing of microorganisms under atmospheric entry heating profile was chosen.
  - Rapid heating capability
  - Controllable cycle profiles
  - Rapid cool-down
  - Optional vacuum exposure

Example exposure profiles

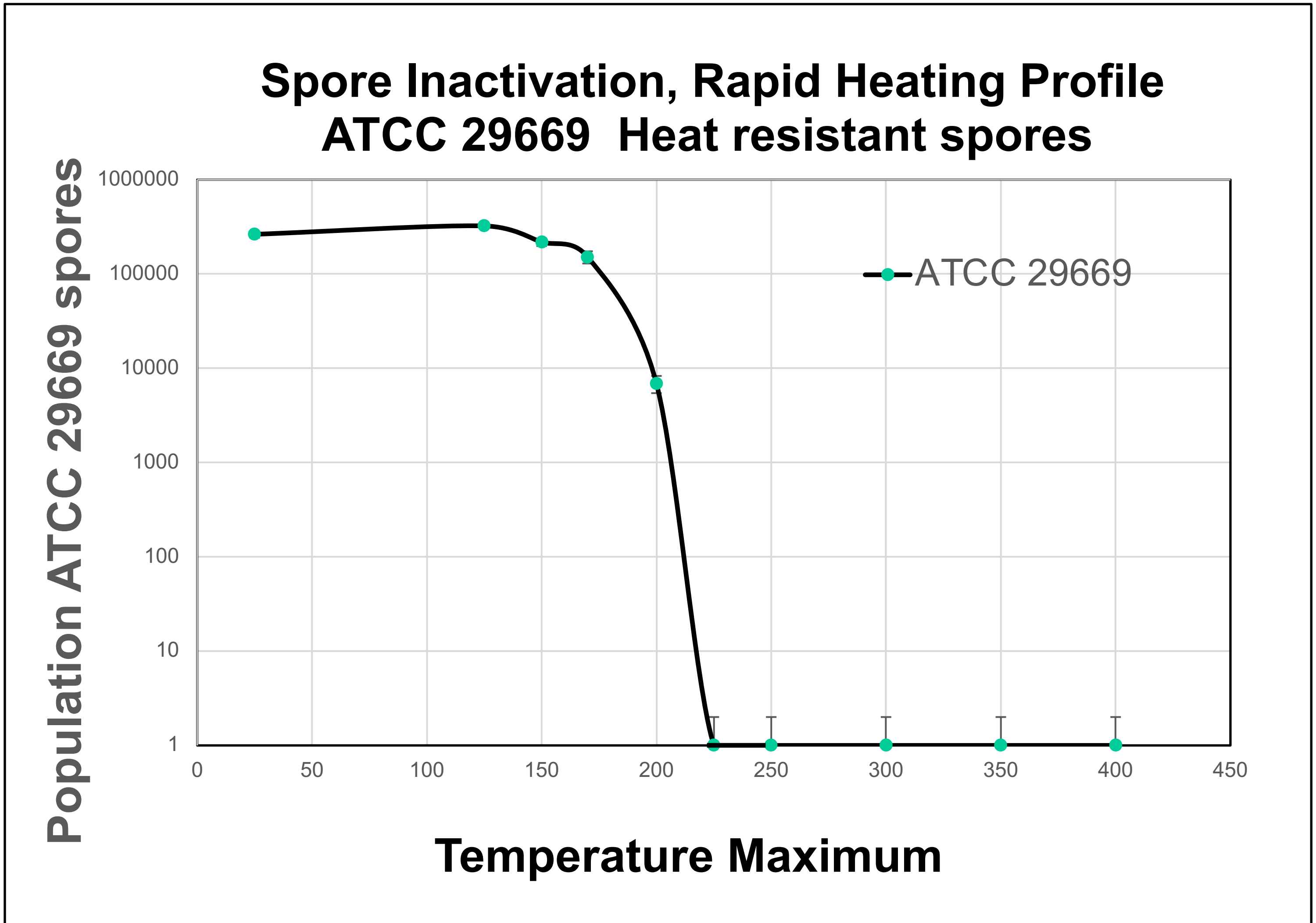




Standard biological indicator bacterial spores are inactivated at temperatures of 200°C



Heat resistant bacterial spores are inactivated at temperatures of 225°C or greater



Summary

- For Mars sample return break-the-chain, rapid heating profiles are sufficient to inactivate Earth bacterial spores at temperatures of 225°C and above.
- This provides a temperature regime that can be used for planning sealing methods by brazing.
- The designed apparatus was able to evaluate *Bacillus* spore inactivation:
  - *Bacillus atrophaeus* spores were inactivated at 200°C and above.
  - *Bacillus sp. ATCC 29669, heat resistant spores were inactivated at 225* °C and above.
- Small Mars-exposed exterior zones of a sample canister could be "sterilized" by the rapid application of heat.
- The designed apparatus reaches target temperatures of 425+ °C and can to be programmed to mimic almost any anticipated temperature profile.

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Acknowledgements

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